Chapter 1
Notch Signaling and Tissue Patterning in Embryology: An Introduction

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Abstract The attention of science first turned to the gene that later earned the name *Notch* over a century ago, when the American scientist John S. Dexter discovered in his laboratory at Olivet College the characteristic notched-wing phenotype (a nick or notch in the wingtip) in mutant fruit flies *Drosophila melanogaster*. At present, it is generally accepted that the Notch pathway governs tissue patterning and many key cell fate decisions and other core processes during embryonic development and in adult tissues. Not surprisingly, a broad variety of independent inherited diseases (including CADASIL, Alagille, Adams-Oliver, and Hajdu-Cheney syndromes) have now convincingly been linked to defective Notch signaling. In the second edition of the book entitled *Notch Signaling in Embryology and Cancer*, leading researchers provide a comprehensive, highly readable overview on molecular mechanisms of Notch signaling (Volume I), and notch's roles in embryology (Vol. II) and cancer (Vol. III). In these introductory pages of Vol. II, we give a short overview on its individual chapters, which are intended to provide both basic scientists and clinicians who seek today's clearest understanding of the broad role of Notch signaling in embryology with an authoritative day-to-day source.

Keywords Notch · Notch signaling · Notch pathway · Embryonic development · Jagged · Delta-like ligand · Cell fate decisions · Tissue patterning

It is now generally accepted that, from sponges, roundworms, *Drosophila melanogaster*, and mice to humans, the Notch pathway governs tissue patterning and many key cell fate decisions and other core processes during embryonic development and in adult tissues (Andersson et al. 2011). When the first edition of *Notch Signaling in Embryology and Cancer* was published by Landes and Springer in 2012 in the

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prestigious series *Advances in Experimental Medicine and Biology*, it was the benchmark on this topic, providing a broad audience (ranging from medical students to basic scientists, physicians, and all other health-care professionals) with up-to-date information in a comprehensive, highly readable format. As the result of the huge mountain of new scientific findings that has been build up in the meantime, which underlines the high biological/clinical relevance of Notch signaling and further unravels their underlying molecular mechanisms, we have decided that it is now the right time to publish an updated and extended version. The second edition of this book has been expanded substantially and has been divided in three separate volumes to include many new chapters. In the different volumes of this book, leading researchers provide a comprehensive, highly readable overview on three important topics related to Notch signaling, namely, the underlying molecular mechanisms that mediate its biological effects (volume I), its role in embryonic development (volume II), and finally its relevance for pathogenesis, progression, prevention, and therapy of cancer (volume III). This second volume summarizes the role of the Notch pathway, which first developed during evolution in metazoans (Gazave et al. 2009; Richards and Degnan 2009) and that was first discovered in a fruit fly (*Drosophila melanogaster*), for tissue patterning and embryonic development. As outlined elsewhere in this book (Reichrath and Reichrath 2020a), the tale that created the name *Notch* began over a century ago, when the American scientist John S. Dexter discovered in his laboratory at Olivet College (Olivet, Michigan, USA) the characteristic notched-wing phenotype (a nick or notch in the wingtip) in mutant fruit flies *Drosophila melanogaster* (Dexter 1914). The alleles causing this phenotype were identified 3 years later at Columbia University (New York City, New York, USA) by another American scientist, Thomas Hunt Morgan (1866–1945) (Morgan 1917), who discovered various mutant loci in the chromosomes of these fruit flies that were associated with several distinct notched-wing phenotypes. Although the majority of them were lethal, these alleles were associated with the characteristic phenotype (a nick in the wingtip and bristle phenotype specifically in female fruit flies), suggesting an association of these alleles with the X chromosome (Morgan 1928). Notably, this discovery and similar investigations that supported the chromosomal theory of inheritance earned Thomas Hunt Morgan in 1933 the Nobel Prize in physiology/medicine. In subsequent decades, despite the extensive research on the *Notch* locus, researchers struggled to identify the function for the *Notch* gene due to the lethality early in embryogenesis and the broad variety of phenotypic consequences of Notch mutants. In the following years, many additional alleles were identified, which were associated with the Notch phenotype. These observations were finally confirmed by cloning and sequencing of the mutant *Notch* locus in the laboratories of Spyros Artavanis-Tsakonas and Michael W. Young, more than half a century later (Wharton et al. 1985; Kidd et al. 1986).

Moreover, a broad variety of independent inherited diseases linked to defective Notch signaling has now been identified, highlighting its clinical relevance. The discovery of these congenital diseases started in 1996 in patients diagnosed with CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, an autosomal dominant hereditary stroke disorder resulting in

vascular dementia) (Joutel et al. 1996), with the linkage analysis-based discovery of heterozygous *NOTCH3* mutations on chromosome 19. In the next year, two laboratories published independently the identification of *JAG1* as the gene within chromosome 20p12 that causes Alagille syndrome (Li et al. 1997; Oda et al. 1997). Since these pioneer investigations, several additional inherited disorders, including Adams-Oliver and Hajdu-Cheney syndromes, have now convincingly been linked to defective Notch signaling. Many of these congenital diseases are rare (prevalences of just a few cases per 100,000), presenting on the one hand severe hurdles to investigating the impact of these genes in humans but demonstrating on the other hand how important Notch pathway components are for human survival. Fortunately, the generation and investigation of knockout mice and other animal models have in recent years resulted in a huge mountain of new information concerning Notch gene function, allowing to separate the role of specific Notch components in human development and disease.

This volume is intended to provide both basic scientists and clinicians who seek today's clearest understanding of the broad role of Notch signaling in embryology with an authoritative day-to-day source. In the first chapter following this introduction, Reichrath and Reichrath give a short overview on the role of Notch signaling for the embryonic development of several selected tissues, namely, the brain, skin, kidneys, liver, pancreas, sensory organs, skeleton, heart, and vascular system (Reichrath and Reichrath 2020a).

In the following chapter, Shahrzad Bahrampour and Stefan Thor discuss the impact of Notch signaling for brain development in detail (Bahrampour and Thor 2020). They point out that, during central nervous system (CNS) development, a complex series of events play out, starting with the establishment of neural progenitor cells, followed by their asymmetric division and formation of lineages and the differentiation of neurons and glia. Studies in the *Drosophila melanogaster* embryonic CNS have revealed that the Notch signal transduction pathway plays at least five different and distinct roles during these events. In their chapter, Bahrampour and Thor review these many faces of Notch signaling and discuss the mechanisms that ensure context-dependent and compartment-dependent signaling. The authors conclude by discussing some outstanding issues regarding Notch signaling in this system, which likely have bearing on Notch signaling in many species.

In the next chapter Wei, Phang, and Jiao underline that the simplicity of the Notch pathway in *Drosophila melanogaster*, in combination with the availability of powerful genetics, makes it an attractive model for studying the fundamental mechanisms of how Notch signaling is regulated and how it functions in various cellular conditions during embryonic development (Wei et al. 2020). In this context, the authors summarize the research advances in *Drosophila* development on the epigenetic mechanisms by which the chromatin assembly factor-1 (CAF-1) regulates Notch signaling activity, which enables Notch to orchestrate different biological inputs and outputs in specific cellular contexts. They convincingly demonstrate that epigenetic regulation of Notch signaling by CAF-1 and other epigenetic regulators plays essential roles in fine-tuning the transcriptional output of Notch signaling to coordinate multicellular organism development. The authors conclude that it

remains an open question as to why and how different epigenetic regulators are involved in mediating different histone modifications status, leading to different transcriptional outputs of either gene repression or gene activation in one specific signal transduction pathway.

Underlining the many facettes of Notch signaling for embryonic development, Makoto Sato and Tetsuo Yasugi discuss in the following chapter the relevance of a combination of Notch-mediated lateral inhibition and epidermal growth factor (EGF)-mediated reaction diffusion for the regulation of proneural wave propagation (Sato and Yasugi 2020). They report that during various biological processes, Notch has to act together with other signaling systems to regulate binary cell fate choice via lateral inhibition resulting in salt-and-pepper pattern formation. However, they emphasize that it is in many cases not clear what happens when Notch is combined with other signaling systems and that mathematical modelling and the use of a simple biological model system will be essential to address this uncertainty. They explain that a wave of differentiation in the *Drosophila* visual center, the "proneural wave," accompanies the activity of the Notch and EGF signaling pathways and that, although all of the Notch signaling components required for lateral inhibition are involved in the proneural wave, no salt-and-pepper pattern is found during the progression of the proneural wave. Instead, Notch is activated along the wave front and regulates proneural wave progression. Makoto Sato and Tetsuo Yasugi ask the question how does Notch signaling control wave propagation without forming a salt-and-pepper pattern? As they point out, a mathematical model of the proneural wave based on biological evidence convincingly demonstrated that Notch-mediated lateral inhibition is implemented within the proneural wave and that the diffusible action of EGF cancels salt-and-pepper pattern formation. They discuss that the results from numerical simulation have been confirmed by genetic experiments in vivo and suggest that the combination of Notch-mediated lateral inhibition and EGF-mediated reaction diffusion enables a novel function of Notch signaling that regulates propagation of the proneural wave. Makoto Sato and Tetsuo Yasugi conclude that similar mechanisms may play important roles in diverse biological processes found in embryonic development and cancer pathogenesis.

In the following chapter, Tetsuichiro Saito convincingly demonstrates that a nucleolar protein, Nepro, is essential for the maintenance of early neural stem cells and preimplantation embryos (Saito 2020). He points out that Notch signaling is required for maintaining neural stem cells (NSCs) in the developing brain and that NSCs have potential to give rise to many neuronal types in the early telencephalon, and the potential decreases as embryonic development proceeds. Tetsuichiro Saito explains that *Nepro*, which encodes a unique nucleolar protein and is activated downstream of Notch, is essential for maintaining NSCs in the early telencephalon. *Nepro* is also expressed at basal levels and required for maintaining the preimplantation embryo, by repressing mitochondria-associated p53 apoptotic signaling. Tetsuichiro Saito points out that Notch signaling also controls dendritic complexity in mitral cells, major projection neurons in the olfactory bulb, and concludes that many steps of neural development involve Notch signaling.

In the following chapter, Sergio Córdoba and Carlos Estella (2020) summarize our present understanding of the role of Notch signaling for leg development in *Drosophila melanogaster*. They explain that the Notch pathway plays diverse and fundamental roles during animal development. One of the most relevant, which arises directly from its unique mode of activation, is the specification of cell fates and tissue boundaries. The development of the leg of *Drosophila melanogaster* is a fine example of this Notch function, as it is required to specify the fate of the cells that will eventually form the leg joints, the flexible structures that separate the different segments of the adult leg. Notch activity is accurately activated and maintained at the distal end of each segment in response to the proximo-distal patterning gene network of the developing leg. Region-specific downstream targets of Notch in turn regulate the formation of the different types of joints. The authors discuss recent findings that shed light on the molecular and cellular mechanisms that are ultimately governed by Notch to achieve epithelial fold and joint morphogenesis. Finally, they briefly summarize the role that Notch plays in inducing the nonautonomous growth of the leg. Overall, this book chapter aims to highlight leg development as a useful model to study how patterning information is translated into specific cell behaviors that shape the final form of an adult organ.

In the next chapter, Nicolas Daudet and Magdalena Żak explain the role of Notch signalling as the multitask manager of inner ear development and regeneration (Daudet and Żak 2020). They point out that Notch signalling is a major regulator of tissue patterning in metazoans, exerting its effects both by lateral inhibition (whereby Notch mediates competitive interactions between cells to limit adoption of a given developmental fate) and by lateral induction (a cooperative mode of action that was originally described during the patterning of the Drosophila wing disc and creates boundaries or domains of cells of the same character). In their chapter, Nicolas Daudet and Magdalena Żak introduce these two signalling modes and explain how they contribute to distinct aspects of the development and regeneration of the vertebrate inner ear, the organ responsible for the perception of sound and head movements. Moreover, Nicolas Daudet and Magdalena Żak discuss in this chapter some of the factors that influence the context-specific outcomes of Notch signalling in the inner ear, and the ongoing efforts to target this pathway for the treatment of hearing loss and vestibular dysfunction.

In the last chapter, Reichrath and Reichrath give a short overview on inherited diseases related to defective Notch signaling, including CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) and Alagille, Adams-Oliver, Hajdu-Cheney, and lateral meningocele syndromes (Reichrath and Reichrath 2020b). They point out that the evolutionary highly conserved Notch pathway governs many cellular core processes including cell fate decisions, although it is characterized by a simple molecular design. Moreover, Notch signaling, which first developed in metazoans, represents one of the most important pathways that govern embryonic development. Consequently, a broad variety of independent inherited diseases linked to defective Notch signaling has now

been identified, including Alagille, Adams-Oliver, and Hajdu-Cheney syndromes, CADASIL, early-onset arteriopathy with cavitating leukodystrophy, lateral meningocele syndrome (LMS), and infantile myofibromatosis. In their review, Reichrath and Reichrath give a brief overview on molecular pathology and clinical findings in congenital diseases linked to the Notch pathway (Reichrath and Reichrath 2020b). Moreover, they discuss the emerging role of Notch as a promising therapeutic target. In this context, it is of interest that in a mouse model of LMS (Notch3$^{tm1.1Ecan}$), cancellous bone osteopenia was no longer detected after intraperitoneal administration of antibodies directed against the negative regulatory region (NRR) of Notch3 (Yu et al. 2019, reviewed in Reichrath and Reichrath 2020b). In that study, anti-Notch3 NRR antibody suppressed expression of Hes1, Hey1, and Hey2 (Notch target genes) and decreased Tnfsf11 (receptor activator of NF kappa B ligand) messenger RNA in Notch3$^{tm1.1Ecan}$ osteoblast cultures (Yu et al. 2019, reviewed in Reichrath and Reichrath 2020b). This study indicates that cancellous bone osteopenia of Notch3$^{tm1.1Ecan}$ mutants can be reversed by anti-Notch3 NRR antibodies, thereby opening new avenues for treatment of bone osteopenia in LMS patients (Yu et al. 2019, reviewed in Reichrath and Reichrath 2020b).

We hope that this volume will provide both basic scientists and clinicians who seek today's clearest understanding of the broad and fascinating role of Notch signaling for the embryonic development with an authoritative day-to-day source.

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